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Expression of catalytically active rat *S*-adenosylmethionine decarboxylase in *Escherichia coli*

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The cDNA coding for rat *S*-adenosylmethionine decarboxylase (AdoMetDC, EC 4.1.1.50) has been cloned into a plasmid expression vector, pKK-223-3, and expressed in *E. coli*. The authenticity of the expressed protein has been demonstrated by reactivity with antibodies specific for rat AdoMetDC, by size analysis on SDS gels visualized with immunotransblots, and, finally, by catalytic activity. The expression of the enzyme results in a decrease in the activity of the bacterial enzyme suggesting the replacement of the bacterial enzyme by the rat AdoMetDC. Similarly, the addition of exogenous spermidine to the growth medium reduces bacterial enzyme activity affecting only marginally the expression of the recombinant protein.

S-Adenosylmethionine decarboxylase; DNA, recombinant; Expression; (*E. coli*, Rat)

1. INTRODUCTION

S-Adenosylmethionine decarboxylase is a key enzyme in polyamine biosynthesis. Its product, decarboxylated AdoMet, serves as an aminopropyl donor in the biosynthesis of spermidine and spermine, two cellular constituents essential for growth and differentiation [1–3]. The rat enzyme is synthesized as an inactive precursor of M_r 37,000. This is converted to two polypeptides of molecular masses about 32,000 and 6500 in a processing reaction which generates the prosthetic pyruvate group [4–6]. Both polypeptides are precipitable by antiserum to AdoMetDC indicating that the smaller subunit does remain as part of the final enzyme [6]. It is not yet known exactly where the cleavage site is located in the rat proenzyme, but the conversion, which is accelerated by the presence of putrescine [7], presumably generates the pyruvate from an internal serine residue. This type of serinolysis reaction has been demonstrated for all of the bacterial enzymes which contain pyruvate in amide linkage [8–11].

The activity of mammalian AdoMetDC is regulated negatively by spermidine and spermine [12]. Polyamine depletion leads to an increase in the amount of AdoMetDC protein caused by both a decreased rate of degradation and an increased rate of synthesis. The increased synthesis has been shown to be partly due to an enhanced translation efficiency [13]. The mechanism by which spermidine and spermine reduce the translational activity of AdoMetDC mRNA is not known. This effect may result from an interaction of the mRNA with the polyamines themselves or with a protein influenced by polyamines [7].

To learn more about the processing of the proenzyme, we expressed rat AdoMetDC cDNA in *E. coli*. The protein produced in *E. coli* had characteristics of the natural rat enzyme including the catalytic activity.

2. MATERIALS AND METHODS

2.1. *Materials*

Escherichia coli strains L87 (supE supF hsdR (r^-m^+) trpR metD tonA), HT551 (Δ(speE-speD)^{a}zad-220::Tn10 panB6 Δ(lacpro)[F′, lacI^{q}ZΔM15, pro$^+$]), HT535 (Δ(lacpro)[F′, lacI^{q}ZΔM15, pro$^+$]), JM101([F′ traD36 pro AB+ lacI^{q}ZΔM15] Δ(pro$^-$lac) mcrA thi lambda$^-$), JM105 ([F′ traD36 proAB+ lacI^{q}ZΔM15]Δ (pro$^-$lac) hsdR4 rpsL sbcB15 thi endA1), JM109 ([F′ traD36 proAB+ lacI^{q}ZΔM15] Δ(lac$^-$proAB) supE44 hsdR17 (r^-m^+) gyrA96 recA1 thi endA1 relA1 lambda$^-$), and NM514 (NM;pop101 hsdR hflA mcrB) were used as indicated. *E. coli* strains HT551 and HT535 were kindly provided by Dr H. Tabor. Restriction enzymes were purchased from Boehringer-Mannheim (FRG) and New England Biolabs (MA). Radiolabeled nucleotides were from Amersham (UK). The expression vector, pKK-223-3, was from Pharmacia.

2.2. *General methods*

Plasmid DNAs were prepared by the alkaline lysis method and purified by centrifugation in cesium chloride-ethidium bromide [14]. Restriction enzyme digests and ligations were carried out as recommended by the suppliers. DNA manipulations and transformation of *E. coli* were performed according to standard procedures [14]. For the sequencing of ligation sites, the chain termination technique was used directly on the pKK-223-3 derivative [15]. The reaction conditions were those outlined in the DNA sequencing protocol supplied with the Sequenase kit (US Biochemical Corp., Cleveland, OH).

2.3. *DNA constructions*

A 471 bp DNA fragment of the pUC19 (pcSAMr1) [15] plasmid

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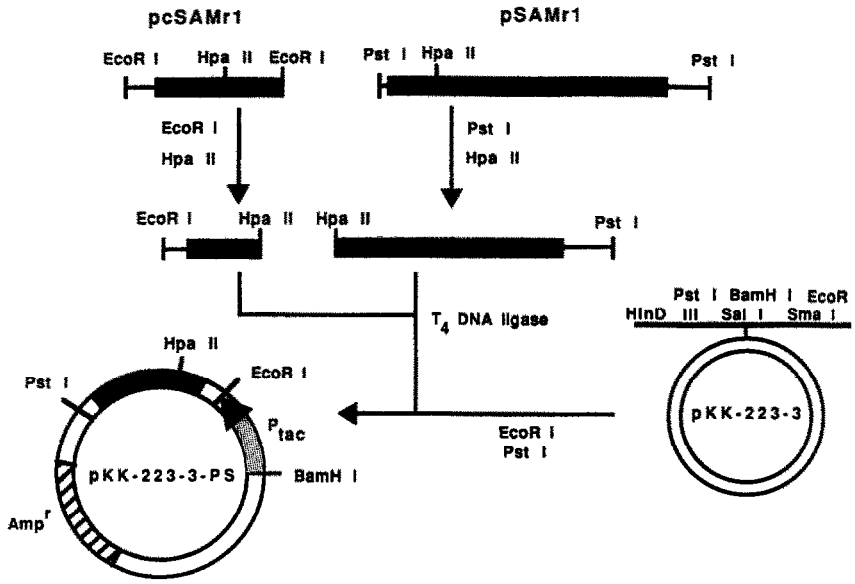

Fig.1. Plasmid construction for expression of rat AdoMetDC in *E. coli*. A 471 bp *Eco*RI-*Hpa*II fragment (pcSAMr1) and a 734 bp *Hpa*II-*Pst*I fragment (pSAMr1), which together contained the entire rat AdoMetDC coding sequence, were ligated into *Eco*RI-*Pst*I cut pKK-223-3 to produce pKK-223-3-PS.

Table 1

Expression of rat AdoMetDC in JM105 cells

	AdoMetDC activity (nmol CO_2/30 min/mg protein)
JM105	0.31
JM105 + IPTG	0.29
JM105 + pKK-223-3	0.46
JM105 + pKK-223-3 + IPTG	0.35
JM105 + pKK-223-3-PS	0.86
JM105 + pKK-223-3-PS + IPTG	9.42

Bacteria were grown at 37°C in the presence of ampicillin (100 μg/ml) in LB broth medium to an absorbance of 0.7 at 600 nm. To induce tac promoter, the culture was supplemented with IPTG to 1 mM and incubated for a further 2 h. The cells (30 ml culture) were harvested by centrifugation and resuspended in 300 μl of 25 mM phosphate buffer, pH 7.5, 2.5 mM DTT, 0.1 mM EDTA. The cells were disrupted by sonication for four 15-s intervals with periods of cooling in ice between, and soluble and insoluble material was separated by centrifugation for 15 min at 14,000 rpm. AdoMetDC activity in the cell lysates was assayed in the presence of putrescine (2.5 mM) but without $MgCl_2$.

Table 2

Expression of rat AdoMetDC in different *E. coli* strains

Strain	AdoMetDC activity (nmol CO_2/30 min/mg protein)	
	+ pKK-223-3 + IPTG	+ pKK-223-3-PS + IPTG
JM101	0.4	3.8
JM109	0.6	3.6
NM514	0.4	8.3
L87	0.4	17.3
AR68	0.3	5.7
HT551	0.2	9.7

Experimental details as described in the text for table 1.

bearing the 5'-end of AdoMetDC cDNA was isolated from a 2% agarose gel following digestion with restriction endonucleases *Eco*RI and *Hpa*II. A cDNA fragment excised covered 383 bp of the coding region and 88 bp of the 5' noncoding region. The 3'-end of the AdoMetDC encoding sequence was excised with *Pst*I and *Hpa*II from the pSV7186 (pSAMr1) [6,15] plasmid followed by a purification of the 734 bp fragment covering 616 bp of the coding region. The resulting fragments were inserted between the *Eco*RI and *Pst*I sites of nKK-223-3 in a tripartite ligation to yield pKK-223-3-PS. This

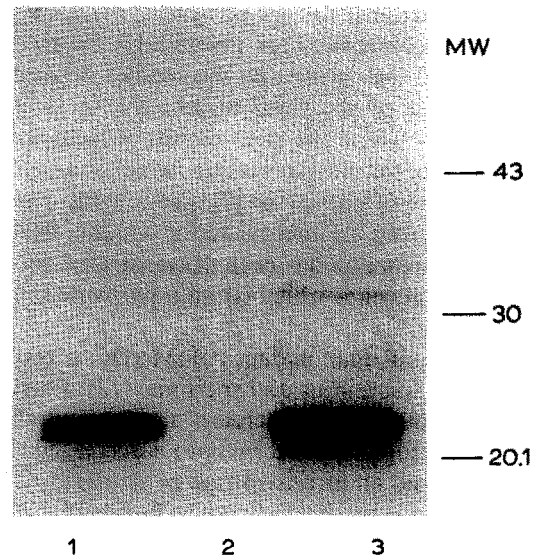

Fig.2. Western blot analysis of *E. coli* (L87) lysates. The samples from the L87 cell extracts were subjected to electrophoresis, transferred to a nitrocellulose membrane and visualized using a specific rabbit antiserum against rat AdoMetDC as described in section 2. (Lane 1) L87 + pKK-223-3-PS; (lane 2) L87 + pKK223-3 + 1 mM IPTG; (lane 3) L87 + pKK-223-3-PS + 1 mM IPTG.

Table 3

Distinction between the bacterial and rat AdoMetDC activities

	AdoMetDC activity (nmol) CO_2/30 min/mg protein	
	Pu	Mg/Pu
L87	0.4	46.2
L87 + pKK-223-3-PS + IPTG	17.9	45.3
L87 + pKK-223-3-PS + IPTG (after immunoprecipitation)	n.d.	23.1

Experimental details as in table 1. AdoMetDC activities were measured with or without $MgCl_2$ addition to the reaction medium (10 mM). n.d., not detectable

strategy is summarized in fig.1. To ascertain the correct reading frame, the ligation sites were sequenced.

2.4. *Expression of AdoMetDC in* E. coli

Recombinant plasmid pKK-223-3-PS was introduced into *E. coli* by calcium chloride-mediated transformation. Bacteria were grown at 37°C in the presence of ampicillin (100 μg/ml) in LB broth medium to an absorbance of 0.7 at 600 nm. To induce the tac promoter, the culture was supplemented with IPTG to 1 mM and incubated for a further 1–2 h. The cells (30 ml culture) were harvested by centrifugation and resuspended in 300 μl of 25 mM phosphate buffer, pH 7.5, 2.5 mM DTT, 0.1 mM EDTA. The cells were disrupted by sonication for four 15-s intervals with periods of cooling in ice between, and soluble and insoluble material was separated by centrifugation for 15 min at 14,000 rpm.

2.5. *Identification of recombinant AdoMetDC*

AdoMetDC from the soluble fraction was assayed by measuring the release of $^{14}CO_2$ from AdoMet-[^{14}COOH] [16] in the presence of putrescine (2.5 mM). The bacterial enzyme can be distinguished from that of the mammalian enzyme by addition of $MgCl_2$ (10 mM) because the bacterial AdoMetDC has a strict requirement of Mg^{2+} for the catalytic activity. Immunoprecipitation was performed by the standard procedure using rabbit antiserum to rat AdoMetDC [17]. SDS-PAGE was performed as described [18]. The protein corresponding to AdoMetDC was detected as described by De Blas and Cherwinski [19] using a specific rabbit antiserum followed by goat anti-rabbit IgG and peroxidase-antiperoxidase. The antiserum was generously provided by Dr A.E. Pegg.

3. RESULTS AND DISCUSSION

The initial expression of AdoMetDC cDNA insert in pKK-223-3 was performed using *E. coli* strain JM105 as a host. As shown in table 1, the cells containing the recombinant plasmid revealed a high AdoMetDC activity when measured in the presence of putrescine but without $MgCl_2$.

To examine whether there was variation in the expression efficiency of rat AdoMetDC between different strains of *E. coli*, pKK-223-3-PS was introduced into the strains JM101, JM109, NM514, L87, a protease-deficient strain AR68 and a AdoMetDC-deficient strain HT551. All the strains tested directed the synthesis of functional rat AdoMetDC. However, under the conditions used, *E. coli* strain L87 produced the highest specific activity for the enzyme (table 2).

To verify the authenticity of enzyme protein, both Western blot analysis and immunoprecipitation of the rat enzyme from L87 lysates were performed. Western blot analysis of *E.* coli lysates (fig.2) demonstrates two proteins, about 32 kDa and 23 kDa respectively, which cross-react with antisera to rat AdoMetDC. The larger protein comigrates with the subunit of the processed rat prostate enzyme. The smaller, more intense band may represent either the degradation product or an incomplete translation product. The fact that this band can be seen in autoradiographs after in vitro translation of rat prostate [7] and SV3T3 poly(A)RNA [20] and, on the other hand, that a 23 kDa protein is present in the lysate of the protease deficient strain AR68 transformed with pKK-223-3-PS (not shown), suggest incomplete translation.

The enzyme activity measurable in the presence of putrescine but not $MgCl_2$ can be completely abolished by immunoprecipitation with the antiserum against the rat enzyme (table 3). These experiments show that the recombinant protein maintains functional activity, and thus present evidence that *E. coli* is able to convert the proenzyme form to catalytically active protein. This suggests a common mechanism for creation of the prosthetic pyruvate group in *E. coli* and in mammals. The recombinant protein is apparently not only catalytically active when measured in vitro but also in vivo since the expression of rat enzyme results in a decrease in the activity of bacterial AdoMetDC (table 3). The same effect

Table 4

The effect of spermidine on the activity of AdoMetDC

	AdoMetDC activity (nmol CO_2/30 min/mg protein)			
Spermidine concentration:	0 mM	0.5 mM	5.0 mM	10 mM
L87	17.1	13.0	5.3	3.3
L87 + pKK-223-3-PS	18.6	19.3	12.8	12.3
HT535	7.2	7.7	2.6	1.0
HT551 + pKK-223-3-PS	7.2	7.0	6.7	6.4

Experimental details as in table 1 except that the cells were grown in the presence of spermidine as indicated and the induction time (1 mM IPTG) was 1 h. AdoMetDC activities were measured in the presence of putrescine (2.5 mM) and $MgCl_2$ (10 mM). *E. coli* strain HT535 (wild type; strain 71.18) was used as the control for the strain HT551 which is a deleted *speED* derivative of strain HT535

can be achieved by adding exogenous spermidine to the growth medium (table 4). The finding that high concentrations of spermidine also decrease the production of the recombinant protein, is obviously due to the well known inhibitory effect of polyamines on polypeptide synthesis at higher concentrations [21].

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